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EXAMINER	
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ART UNIT	**PAPER NUMBER**
1652	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/612,779	DENG ET AL.	
	Examiner	Art Unit	
	Delia M. Ramirez	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 May 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-4,7-14,17-61 and 207-226* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-4,7-14,17-61 and 207-226* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *01 July 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *2/7/05,4/22/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Status of the Application

Claims 1-4, 7-14, 17-61, 207-226 are pending.

Applicant's amendment canceling claims 5-6, 15-16, 62-206, and addition of claims 213-226 as submitted in a communication filed 5/22/2006 is acknowledged.

Applicant's election with traverse of Group III, claims 1-61, 207-212 drawn in part to a method to produce glucosamine or N-acetylglucosamine comprising culturing a microorganism which has at least one genetic modification that increases the activity of glucosamine-6-phosphate acetylransferase, wherein said microorganism is transformed with a polynucleotide encoding the polypeptides of SEQ ID NO: 30 and 6 in a communication filed 5/22/2006 is acknowledged.

Applicant traverses the restriction requirement as it relates to Groups I-XXX on the grounds that a through search of claims 1-4 is sufficient to examine the subject matter of all the inventions in these groups because these claims are not limited to a particular sequence. Applicant submits that claims 1-4 are linking claims and are not limited to a particular sequence, a particular combination of modifications, or to transformation of the microorganism. In addition, Applicant submits that since claim 1 only requires a genetic modification which increases the activity of glucosamine-6-phosphate acetyltransferase, restriction to every possible combination of specific sequences is unduly restrictive. Applicant submits that examination of claim 1 should be sufficient to examine the dependent embodiments, thus reducing the number of groups to three. Furthermore, Applicant indicates that SEQ ID NO: 6, 8, 10, 12 and 14 are variants of the protein of SEQ ID NO: 2 with a small number of substitutions. Therefore, a search which includes all these sequences can be made without serious burden.

Applicant's arguments have been fully considered. The Examiner agrees that claim 1 is a linking claim for Groups I-XXX and that claims 1-4 are not limited to a particular sequence, combination or transformation of a microorganism. While claim 1 was not specifically stated in the restriction

requirement as a linking claim, the Examiner included generic linking claim 1 as well as generic claims 2-4, 9-14, 19, 21-61, 207-212 in Groups I-XXX. As such, the full scope of these generic claims will be examined.

Arguments indicating that the restriction should be withdrawn because SEQ ID NO: 4, 6, 8, 10, 12 and 14 are variants of SEQ ID NO: 2 with minor substitutions have been found persuasive. While claim 1 links properly divisible inventions as set forth in MPEP 809.03 for the reasons of record, upon aligning these sequences and reviewing the specification (page 28), it has been found that these variants of SEQ ID NO: 2 have no more than 4 amino acid substitutions with respect to SEQ ID NO: 2. SEQ ID NO: 10 and 12 appear to be identical. A search of SEQ ID NO: 6 is deemed co-extensive with regard to SEQ ID NO: 2, 4, 8, 10, 12 and 14. Thus, the restriction requirement with regard to those groups which require SEQ ID NO: 30 and SEQ ID NO: 2, 4, 6, 8, 10, 12, and 14 (i.e. Groups I-VII) is hereby withdrawn.

The restriction requirement between the remaining linked inventions is subject to the nonallowance of the linking claim(s), claim 1. Upon the indication of allowability of the linking claim(s), the restriction requirement as to the remaining linked inventions shall be withdrawn and any claim(s) depending from or otherwise requiring all the limitations of the allowable linking claim(s) will be rejoined and fully examined for patentability in accordance with 37 CFR 1.104.

The requirement is still deemed proper and therefore is made FINAL.

Neither linking claim 1 nor generic claims 2-4, 9-14, 19, 21-61, 207-212, 218 are allowable at this time. Claims 7-8, 17, 18, 20 and new claims 213-216 are directed in part to the elected invention and will be examined to the extent they encompass the elected invention. Claims 1-4, 9-14, 19, 21-61, 207-212, 217-226 and claims 7-8, 17, 18, 20 and 213-216 in part are at issue and are being examined herein.

Priority

1. Acknowledgment is made of a claim for domestic priority under 35 U.S.C. 119(e) to provisional application No. 60/393,348 filed on 07/01/2002.

Information Disclosure Statement

2. The information disclosure statements (IDS) submitted on 2/7/2005 and 4/22/2004 are acknowledged. The submissions are in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statements are being considered by the examiner.

Drawings

3. The drawings submitted 7/1/2003 have been reviewed and are accepted by the Examiner.

Claim Objections

4. Claim 58 is objected to due to the recitation of "c) dephosphorylating....; and d) dephosphorylating.....N-acetylglucosamine e) treating....". The term "and" should be placed between items d) and e). Also, a punctuation mark separating d) and e) should be inserted in the claim. Appropriate correction is required.

5. Claim 207 is objected to due to the recitation of "inducing transcription of the nucleic acid sequence" for the following reasons. As known in the art, a sequence is a graphical representation of the order in which nucleic acids are arranged in a nucleic acid molecule. Therefore, it is the nucleic acid and not its sequence what is induced for transcription. The Examiner has understood that the term refers to induction of the nucleic acid. However, the claim should be amended to clearly indicate that induction applies to the nucleic acid. Appropriate correction is required.

Claim Rejections - 35 USC § 112, Second Paragraph

6. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

7. Claims 7, 17, 58-59, 207-217, 219-221, 223-226 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

8. Claims 7, 17, 213-217, 219 (claims 220-221, 223-226 dependent thereon) are indefinite in the recitation of "at least about" for the following reasons. The use of this language is contradictory because the term "about" can be interpreted as "less than" whereas the term "at least" is synonym of "no less than". For examination purposes, it will be assumed that the claims read "at least". Correction is required.

9. Claim 58 is indefinite in the recitation of the term "b) recovering a product selected from the group consisting of ....from the organism...." for the following reasons. Claim 58 is directed in part to the method of claim 1 wherein the method further comprises recovering specific products from the organism. Claim 1 already contains a step for collecting a genus of products wherein the genus encompasses the products to be recovered in b). Thus, it is unclear as to how claim 58 further limit claim 1 since collecting the product would imply recovering the product. For examination purposes, no patentable weight will be given to step b) in claim 58. Correction is required.

10. Claim 59 is indefinite in the recitation of "the method of claim 54, wherein step (e) comprises hydrolyzing..." because there is no antecedent basis for step (e) in claim 54. For examination purposes, it will be assumed that claim 59 depends on claim 58. Correction is required.

11. Claim 207 (claims 208-212 dependent thereon) is indefinite in the recitation of "ii) inducing transcription....by addition of lactose to the fermentation medium in the absence of adding glucose to the medium; iii) fermenting the microorganism after step (ii) in the presence of glucose..." for the following

reasons. It appears that in step ii) the claim requires no addition of glucose while induction takes place although residual glucose in the culture is allowed. Step iii) requires the presence of glucose. The term "after step (ii)" is unclear because one cannot determine whether the term implies immediately after induction starts, or if the term implies after the entire induction process is over. As known in the art, while some types of inductions are short-lived (e.g., temperature induction), chemical induction with a chemical which is not metabolized by the cell does not stop once the inducer is added to the culture (e.g., IPTG). Thus, if the term "after step (ii)" is intended to mean "after the induction process is over", it is unclear as to how step iii) can be carried out when induction is not temporary since step ii) requires no additional glucose, and the remaining glucose will eventually be consumed. For examination purposes, no patentable weigh will be given to step (iii). Correction is required.

12. Claim 208 (claim 209 dependent thereon) is indefinite in the recitation of "trace elements is added to step (iii) of fermenting" for the following reasons. While step (iii) is a fermentation step, as written, the term "step (iii) of fermenting" implies that the fermentation step comprises a third step. However, there is no third step within the fermentation step. For examination purposes, it will be assumed that the claim reads "trace elements is added to step (iii)". Correction is required.

13. Claim 210 is indefinite in the recitation of "wherein step (ii) comprises growing the microorganism..." because step (ii) of claim 207 is an induction step. Therefore, it is unclear as to how the claim further limits claim 207. For examination purposes, the Examiner will interpret the claim to recite "wherein step (i)". Correction is required.

Claim Rejections - 35 USC § 112, First Paragraph

14. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

15. Claims 1-4, 7-14, 17-61, 207-226 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

As stated in MPEP 2111.01, during examination, the claims must be interpreted as broadly as their terms reasonably allow. While the term "the amino acid sequence of SEQ ID NO: X" clearly indicates that the amino acid sequence contains all of SEQ ID NO: X, the term "an amino acid sequence of SEQ ID NO: X" as recited in claims 46, 50, 53, 220-222 can be interpreted as "an amino acid sequence within SEQ ID NO: X" (i.e., not all of SEQ ID NO: X). Thus, in the instant case, the Examiner has broadly interpreted the term "an amino acid sequence of SEQ ID NO:X" to encompass a fragment of at least 2 amino acids of SEQ ID NO:X. It is also noted that the specification discloses the term "inactivation" as encompassing any method which would lead to a non-functional gene/gene product. This would encompass, for example, chemical and biological inhibitors of gene expression, chemical and biological inhibitors of enzymatic activity, modifications in the promoter region of the gene to be inactivated, and modifications in any gene which encodes a protein that acts as a transcriptional modulator of the gene to be inactivated. In view of this interpretation, claims 1-4, 7-12, 21-61, 213-214 are directed in part a method to produce glucosamine or N-acetylglucosamine by fermentation wherein said method requires culturing a microorganism which comprises (1) any genetic modification which would result in an increase in the activity of a genus of glucosamine-6-phosphate acetyltransferases by several methods, including but not limited to, increased enzymatic activity by structural modifications to the enzyme, reduction in feedback inhibition of the acetyltransferase by N-acetylglucosamine-6-phosphate, and increased affinity of the acetyltransferase for glucosamine-6-phosphate, (2) any genetic modification which would result in reduction or elimination of the activity of a genus of glucosamine-6-phosphate deaminases, (3) any genetic modification which would result in an increase in the activity of a

genus of phosphoglucoisomerases, (4) any genetic modification which would result in an increase in the activity of a genus of glutamine synthetases, (5) any modification which would result in inactivation of a genus of phosphofructokinases, (6) any genetic modification which would result in an increase in the activity of a genus of glucose-6-phosphate dehydrogenases, and (7) any modification which would result in inactivation of a genus of genes encoding any enzyme associated with glycogen synthesis. Claims 13-14, 17-20, 215-217 are directed in part to a method to produce glucosamine or N-acetylglucosamine by fermentation wherein said method requires culturing a microorganism which comprises any genetic modification which would result in (1) an increase in the activity of a genus of glucosamine-6-phosphate acetyltransferases, and (2) an increase in the activity of a genus of glucosamine-6-phosphate synthases. Claims 207-212 are directed to a fermentation process for the production of glucosamine wherein a microorganism has been transformed with a genus of nucleic acids encoding glucosamine-6-phopshate synthases. Claims 218-226 are directed in part to a method to produce glucosamine or N-acetylglucosamine by culturing a microorganism which expresses (1) a genus of nucleic acids encoding glucosamine-6-phosphate acetyltransferases, and a genus of nucleic acids encoding glucosamine-6-phosphate synthases which have reduced product feedback inhibition, or (2) nucleic acids encoding proteins comprising any fragment of SEQ ID NO: 30 and 6, wherein said method also comprises inactivation by any means of a genus of phosphofructokinases or the *E. coli* pfkA gene product, and inactivation by any means of the *E. coli* nagA, nagB, nagE and manXYZ gene products. See Claim Rejections under 35 USC 112, second paragraph for claim interpretation.

In *University of California v. Eli Lilly & Co.*, 43 USPQ2d 1938, the Court of Appeals for the Federal Circuit has held that "A written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula, [or] chemical name,' of the claimed subject matter sufficient to distinguish it from other materials". As indicated in MPEP § 2163, the written description requirement for a claimed genus may be satisfied

through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show that Applicant was in possession of the claimed genus. In addition, MPEP § 2163 states that a representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.

The claims require any number of genetic and non-genetic modifications which would result in either an increase/reduction in activity of a genus of enzymes or the complete/partial inactivation of a genus of genes or the proteins they encode, which the specification fails to describe. While the claims encompass modifications such as mutations in the regulatory regions of a gene to increase/decrease transcription, mutations in the coding region of an enzyme to enhance or decrease its enzymatic activity, mutations in the coding region of an enzyme such that its affinity for its substrate increases or it has reduced product feedback inhibition, the addition of inhibitors of enzymatic activity or inhibitors of gene transcription, expression of genes which encode proteins that are transcription modulators of the target genes, and mutations in genes that encode transcriptional modulators, the specification fails to disclose which are the mutations that need to be made in the regulatory or coding regions of the recited genes, the structure of inhibitors/enhancers of the recited enzymatic activity or gene transcription, the structure of genes which encode transcription regulators of the recited genes, or the mutations which would modulate the expression of genes encoding transcription regulators of the recited genes. There is no additional information regarding the genus of modifications required beyond inactivation of a gene and its gene product by introducing deletions or insertions in the target gene, and increased enzymatic activity by

overexpression of the target gene achieved by increasing the gene's copy number or by using a strong heterologous promoter.

In addition to a genus of modifications which have not been described, the claims require a genus of nucleic acids and proteins which is structurally unrelated. A sufficient written description of a genus of polynucleotides and proteins may be achieved by a recitation of a representative number of polynucleotides and proteins defined by their nucleotide or amino acid sequence or a recitation of structural features common to members of the genus, which features constitute a substantial portion of the genus. However, in the instant case, either there is no structural feature recited which is representative of all the members of the genus of nucleic acids and proteins required in the claimed invention, or the structural feature as interpreted, i.e., any fragment of SEQ ID NO: X, does not constitute a substantial portion of the genus as the remainder of any polypeptide comprising said structural elements is completely undefined and the specification does not define the remaining structural features for members of the genus to be selected. There is no information as to a correlation between the structures disclosed/known in the art and the required activities. Furthermore, while one could argue that the structures of those nucleic acids/proteins disclosed in the specification and the prior art are representative of all members of the genus of nucleic acids/proteins required, such that the claimed invention is adequately described, it is noted that the art teaches several examples of how even small variations in structure can lead to functional variation. For example, Witkowski et al. (Biochemistry 38:11643-11650, 1999) teaches that one conservative amino acid substitution transforms β-ketoacyl ketoacyl synthase into a malonyl decarboxylase and completely eliminates β-ketoacyl activity. Seffernick et al. (J. Bacteriol. 183(8):2405-2410, 2001) teaches that two naturally occurring *Pseudomonas* enzymes having 98% amino acid sequence identity catalyze two different reactions: deamination and dehalogenation, therefore having different function. Therefore, since minor structural changes may result in changes affecting function, and no additional information correlating structure with activity has been provided, one cannot

reasonably conclude that the known structures are representative of all the nucleic acids required in the claimed invention.

Due to the fact that the specification only discloses a few species of the genus of genetic/non-genetic modifications and polynucleotides/proteins recited, and the lack of description of any additional species by any relevant, identifying characteristics or properties, one of skill in the art would not recognize from the disclosure that Applicant was in possession of the claimed invention.

16. Claims 1-4, 7-14, 17-61, 207-226 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a fermentation method to produce glucosamine or N-acetylglucosamine which comprises culturing an *E. coli* cell transformed with (1) a nucleic acid encoding the polypeptide of SEQ ID NO: 30, and (2) a nucleic acid encoding the polypeptides of SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14, wherein the *E. coli* cell further comprises inactivating deletions in the pfkA, nagA, nagB, nagE and manXYZ genes, does not reasonably provide enablement for a fermentation method to produce glucosamine or N-acetylglucosamine by culturing a microorganism which has (1) any genetic modification that would result in (i) increased activity of any glucosamine-6-phosphate acetyltransferase, glucosamine-6-phosphate synthase, phosphoglucoisomerase, glutamine synthase, or glucose-6-phosphate dehydrogenase, or (ii) decreased activity of any microbial glucosamine-6-phosphate deaminase, and (2) any modification that would result in inactivation of (i) phosphofructokinase, or (ii) any enzyme associated with glycogen synthesis. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2nd 1400 (Fed. Cir. 1988)) as follows: (1) quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence

and absence of working examples, (4) the nature of the invention, (5) the state of prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breath of the claims. The factors which have lead the Examiner to conclude that the specification fails to teach how to make and/or use the claimed invention without undue experimentation, are addressed in detail below.

The breath of the claims. Claims 1-4, 7-14, 17-61, 207-226 are so broad as to encompass (1) a method to produce glucosamine or N-acetylglucosamine by fermentation wherein said method requires culturing a microorganism which comprises (i) any genetic modification which would result in an increase in the activity of any glucosamine-6-phosphate acetyltransferase, (ii) any genetic modification which would result in a reduction in the activity of any glucosamine-6-phosphate deaminase, (iii) any genetic modification which would result in an increase in the activity of any phosphoglucoisomerase, (iv) any genetic modification which would result in an increase in the activity of any glutamine synthetase, (v) any modification which would result in inactivation of any phosphofructokinase, (vi) any genetic modification which would result in an increase in the activity of any glucose-6-phosphate dehydrogenase, and (vii) any modification which would result in inactivation of any gene encoding any enzyme associated with glycogen synthesis, (2) a method to produce glucosamine or N-acetylglucosamine by fermentation wherein said method requires culturing a microorganism which comprises any genetic modification which would result in (i) an increase in the activity of any glucosamine-6-phosphate acetyltransferase, and (ii) an increase in the activity of any glucosamine-6-phosphate synthase, (3) a fermentation process for the production of glucosamine wherein a microorganism has been transformed with a nucleic acid encoding any glucosamine-6-phopshate synthase, (4) a method to produce glucosamine or N-acetylglucosamine by culturing a microorganism/*E. coli* cell which expresses (i) a nucleic acid encoding any glucosamine-6-phosphate acetyltransferase, and a nucleic acid encoding any glucosamine-6-phosphate synthase which has reduced product feedback inhibition, or (ii) nucleic acids encoding proteins comprising any fragment of SEQ ID NO: 30 and 6, wherein said method also

comprises inactivation by any means of a genus of phosphofructokinases or the *E. coli* pfkA gene product, and inactivation by any means of the *E. coli* nagA, nagB, nagE and manXYZ gene products. See Claim Rejections under 35 USC 112, second paragraph, for claim interpretation and Claim Rejections under 35 USC 112, first paragraph, written description, for discussion of scope.

The enablement provided is not commensurate in scope with the claims due to the potentially large number of genetic and non-genetic modifications of unknown nature required in any microbial organism such that the activity of the recited enzymes is increased/decreased/inactivated, as well as the extremely large number of genes, proteins and modulators of unknown structure required in the claimed method. In the instant case, the specification enables a fermentation method to produce glucosamine or N-acetylglucosamine which comprises culturing an *E. coli* cell transformed with (1) a nucleic acid encoding the polypeptide of SEQ ID NO: 30, and (2) a nucleic acid encoding the polypeptides of SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14, wherein the *E. coli* cell further comprises inactivating deletions in the pfkA, nagA, nagB, nagE and manXYZ genes

The amount of direction or guidance presented and the existence of working examples. The specification discloses as a working example a method to produce glucosamine or N-acetylglucosamine with an *E. coli* host cell transformed such that (1) it overexpresses a nucleic acid encoding the glucosamine-6-phosphate acetyltransferase of SEQ ID NO: 30 and a nucleic acid encoding variants of the *E. coli* glucosamine-6-phosphate synthase of SEQ ID NO: 2 (i.e., SEQ ID NO: 4, 6, 8, 10, 12, 14) which have reduced product feedback inhibition, by induction with IPTG, and (2) it has an inactivating deletion in the *E. coli* pfkA gene. However, the specification fails to disclose additional genetic and non/genetic modifications required in any microorganism such that the activity of the enzymes recited is increased/decreased/inactivated beyond inactivating deletions/insertions in a gene, overexpression of a gene by increasing its copy number, or by placing it under the control of a strong heterologous promoter, and expression of nucleic acids encoding specific variants of the *E. coli* glucosamine-6-phosphate

synthase of SEQ ID NO: 2 which have decreased product feedback inhibition. Furthermore, the specification fails to disclose the structures of all the genes/proteins which are subjected to the modifications encompassed by the claims. In addition, it is noted that while the specification discloses a method to produce N-acetylglucosamine by overexpression of a nucleic acid encoding an N-acetylglucosamine-6-phosphate acetyltransferase, there is no disclosure of a method to produce glucosamine solely by overexpressing a nucleic acid encoding an N-acetylglucosamine-6-phosphate acetyltransferase. See, for example, page 156, lines 18-21. As shown in Figure 2, glucosamine-6-phosphate acetyltransferase catalyzes the conversion of glucosamine-6-phosphate to N-acetylglucosamine-6-phosphate, thus overexpression of glucosamine-6-phosphate acetyltransferase would result in more of the precursor of glucosamine (glucosamine-6-phosphate) to be redirected to the synthesis of N-acetylglucosamine.

The state of prior art, the relative skill of those in the art, and the predictability or unpredictability of the art. There is no teaching in the art as to all the genetic and non-genetic modifications required in any microorganism such that the activity of the enzymes recited in the claims is increased/decreased/inactivated. While encompassed by the claims, neither the specification nor the prior art teach or disclose which are the mutations that need to be made in the regulatory or coding regions of the recited genes, the structure of chemical/biological inhibitors of the recited enzymatic activity or gene transcription, the structure of genes which encode transcription regulators of the recited genes, the structure of genes which encode enzymatic activity enhancers, or the mutations which would modulate the expression of genes encoding transcription regulators of the recited genes.

The amino acid sequence of a protein determines the structural and functional properties of that protein. In the instant case, neither the specification nor the art provide a correlation between structure and activity such that one of skill in the art can envision the structure of all the enzymes recited in the claims, the structural modifications which would be required to obtain enzymes having the desired

functional characteristics, or the structure of all the proteins which can be transcriptional modulators or inhibitors/enhancers of the enzymes recited in the claims. The art clearly teaches the high level of unpredictability with regard to the effect of structural changes in a protein's activity when no guidance/knowledge as to which amino acids are required for activity has been provided. At the time of the invention there was a high level of unpredictability associated with altering a polypeptide sequence with an expectation that the polypeptide will maintain the desired activity. For example, Branden et al. (Introduction to Protein Structure, Garland Publishing Inc., New York, page 247, 1991) teach that (1) protein engineers are frequently surprised by the range of effects caused by single mutations that they hoped would change only one specific and simple property in enzymes, (2) the often surprising results obtained by experiments where single mutations are made reveal how little is known about the rules of protein stability, and (3) the difficulties in designing *de novo* stable proteins with specific functions. The teachings of Branden et al. are further supported by the teachings of Witkowski et al. (Biochemistry 38:11643-11650, 1999) and Seffernick et al. (J. Bacteriol. 183(8):2405-2410, 2001) already discussed above, where it is shown that even small amino acid changes result in enzymatic activity changes.

The quantity of experimentation required to practice the claimed invention based on the teachings of the specification. While methods of generating or isolating variants of a polypeptide/polynucleotide were known in the art at the time of the invention, as well as methods to overexpress a gene by increasing its copy number or by placing it under the control of a strong heterologous promoter, and methods to disrupt a gene by insertions or deletions, it was not routine in the art to screen by a trial and error process for (1) all proteins to find those having the recited enzymatic activities, (2) the essentially infinite number of mutations within the regulatory or coding region of a gene to determine which mutations achieved the desired result, or (3) all chemical/biological compounds which would inhibit/enhance the recited enzymatic activities or would inhibit/enhance the transcription of the genes encoding the recited enzymes. In the absence of (1) a correlation between structure and function,

(2) some guidance as to which are the structural changes that would result in increased/reduced enzymatic activity or increased/reduced transcription, (3) some guidance as to the structure of the chemical/biological enhancers/inhibitors of all the enzymes recited, (4) some guidance as to which are the chemical/biological enhancers/inhibitors of transcription of the genes encoding the recited enzymes, one of skill in the art would have to (1) test an extremely large number of proteins to determine which ones have the required enzymatic activity, (2) test an extremely large number of mutations to determine which ones result in an increase/decrease/inactivation of the recited enzymes, and (3) test an infinite number of compounds/biologicals to determine which ones enhance/inhibit the enzymatic activity recited or the translation of genes encoding such enzymes.

Therefore, taking into consideration the extremely broad scope of the claims, the lack of guidance, the amount of information provided, the lack of knowledge about a correlation between structure and function, and the high degree of unpredictability of the prior art in regard to structural changes and their effect on function, one of ordinary skill in the art would have to go through the burden of undue experimentation in order to practice the claimed invention. Thus, Applicant has not provided sufficient guidance to enable one of ordinary skill in the art to make and use the invention in a manner reasonably correlated with the scope of the claims.

Conclusion

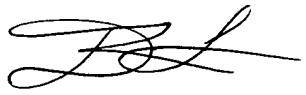
17. No claim is in condition for allowance.

18. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PMR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should

you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

19. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Delia M. Ramirez whose telephone number is (571) 272-0938. The examiner can normally be reached on Monday-Friday from 8:30 AM to 5:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Ponnathapura Achutamurthy can be reached on (571) 272-0928. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (571) 272-1600.



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DR
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